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Influence of Strain, Dose of Virus, and Age at Inoculation on Subgroup J Avian Leukosis Virus Persistence, Antibody Response, and Oncogenicity in Commercial Meat-Type Chickens

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SUMMARY. The effects of viral strain, viral dose, and age of bird at inoculation on subgroup J avian leukosis virus (ALV J) persistence, neutralizing antibody (VNAb) response, and tumors were studied in commercial meat-type chickens. Chickens were inoculated on the fifth day of embryonation (5 ED) or on day of hatch (DOH) with either 100 or 10,000 50% tissue-culture infective dose ($TCID_{50}$) of one of three ALV J strains, namely ADOL Hc1, ADOL 6803, or ADOL 4817. At 1, 3, 7, 11, 15, 19, 23, 27, and 32 wk posthatch, chickens were examined for ALV J viremia and VNAb against the inoculated strain of ALV J. A high incidence (83%–100%) of ALV J persistence was observed in all treatment groups. Development of VNAb did not always lead to viremia-free status; even though 18% of the chickens developed VNAb, only 4% were able to clear viremia. The viral strain, dose, and age of bird at inoculation seemed to have an effect on the incidence of VNAb; however, the differences were statistically significant in only some treatment groups. Chickens infected with ADOL 6803 had higher incidence of VNAb than chickens infected with ADOL Hc1 and ADOL 4817 ($P < 0.05$ in groups 5 ED at 100 $TCID_{50}$ and DOH at 10,000 $TCID_{50}$). There was a trend in all groups inoculated with 100 $TCID_{50}$ to have higher incidence of VNAb than that of groups inoculated with 10,000 $TCID_{50}$ (ADOL 6803 at 5 ED and ADOL 4817 at DOH [$P < 0.05$]; ADOL Hc1 at DOH [$P < 0.08$]). In most treatment groups (ADOL Hc1 at 100 and 10,000 $TCID_{50}$, ADOL 6803 at 10,000 $TCID_{50}$, and ADOL 4817 at 100 $TCID_{50}$), chickens inoculated at DOH had higher incidence of VNAb than that of chickens inoculated at 5 ED (ADOL 6803 at 10,000 $TCID_{50}$ [$P < 0.05$], ADOL Hc1 at 100 $TCID_{50}$ [$P < 0.08$]). Incidence of ALV J-induced tumors and tumor spectrum were influenced by viral strain, age at inoculation, and VNAb response.

RESUMEN. Efecto de la cepa, dosis del virus y edad del ave al momento de la inoculación en la persistencia del virus de leucosis aviar del subgrupo J, la respuesta de anticuerpos y la oncogenicidad en aves de engorde comerciales.

Se estudió la influencia de la cepa, la dosis del virus y de la edad del ave al momento de la inoculación en la persistencia del virus de leucosis aviar del subgrupo J, así como en la respuesta de anticuerpos neutralizantes y la formación de tumores en aves comerciales de la línea de engorde. Las aves se inocularon al quinto día de la etapa embrionaria o el día del nacimiento con 100 ó 10,000 dosis infecciosa 50% para cultivo celular (por sus siglas en Inglés $TCID_{50}$) con una de tres cepas del virus de leucosis aviar del subgrupo J denominadas ADOL Hc1, ADOL 6803, ó ADOL 4817. Las aves se examinaron en las semanas 1, 3, 7, 11, 15, 19, 23, 27 y 32 posteriores al nacimiento, para detectar viremia y la presencia de anticuerpos neutralizantes contra la cepa inoculada del virus de leucosis aviar del subgrupo J. Se observó una alta persistencia para el virus de leucosis aviar del subgrupo J en todos los grupos experimentales (83% a 100%). El desarrollo de anticuerpos neutralizantes no siempre condujo a un estado libre de viremia, pues aunque el 18% de las aves desarrollaron anticuerpos neutralizantes, sólo el 4% fue capaz de eliminar la viremia. La cepa del virus, la dosis y la edad de las aves al momento de la inoculación aparentemente tienen un efecto en la incidencia de anticuerpos neutralizantes, sin embargo, las diferencias fueron estadísticamente significativas solo en algunos grupos. Las aves infectadas con ADOL 6803 mostraron una mayor incidencia de anticuerpos neutralizantes que las aves infectadas con ADOL Hc1 y ADOL 4817 ($P < 0.05$ en grupos inoculados al quinto día de la etapa embrionaria con 100 $TCID_{50}$ y en aves infectadas el día del nacimiento con 10,000 $TCID_{50}$). Se demostró una tendencia hacia una mayor incidencia de anticuerpos neutralizantes para todos los grupos inoculados con 100 $TCID_{50}$ en comparación con los grupos inoculados con 10,000 $TCID_{50}$ (ADOL 6803 inoculados al quinto día de la etapa embrionaria y ADOL 4817 inoculados al nacimiento [$P < 0.05$]; ADOL Hc1 [$P < 0.08$]). En la mayoría de los grupos experimentales, las aves inoculadas el día del nacimiento (ADOL Hcl a 100 y 10,000 $TCID_{50}$ ADOL 6803 a 10,000 $TCID_{50}$ y ADOL 4817 a 100 $TCID_{50}$), mostraron una mayor incidencia de anticuerpos neutralizantes que las aves inoculadas al día 5 de la etapa embrionaria (ADOL 6803 a 10,000 $TCID_{50}$ [$P < 0.05$], ADOL Hcl a 100 $TCID_{50}$ [$P < 0.08$]). La incidencia de tumores inducidos por el virus de leucosis aviar subgrupo J y el espectro de los tumores se vió influenciado por la cepa del virus, la edad de inoculación y la respuesta de anticuerpos neutralizantes.

Key words: subgroup J avian leukosis virus, virus persistence, effect of virus strain and dose, age at inoculation, tolerance, antibody, tumors, commercial meat-type chickens

Abbreviations: ADOL = Avian Disease and Oncology Laboratory; ALV = avian leukosis virus; ALV J = subgroup J avian leukosis virus; BSL-2 = biosecurity level 2; DOH = day of hatch; 5 ED = fifth day of embryonation; ELISA = enzyme-linked immunosorbent assay; gsa = group-specific antigen; LL = lymphoid leukosis; $TCID_{50}$ = 50% tissue-culture infective dose; VNAb = neutralizing antibody against the inoculated parental virus

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Subgroup J avian leukosis virus (ALV J) causes a variety of neoplasms, mainly of myeloid lineage along with a varied incidence of renal neoplasms, erythroblastosis, histiocytic sarcomas, hemangiomas, and connective tissue neoplasms (7). ALV J also causes several non-neoplastic conditions, such as depression in body weight and increased feed conversion ratios, resulting in production losses to the poultry producer (7). ALV J infection is largely controlled by identification and eradication of chickens positive for antibody or viremia, depending on the eradication protocol followed by the primary breeder. Overall, the broiler breeder industry has been successful in controlling ALV J, except for the infrequent incidence of ALV J viremia or antibodies in some flocks. As with other retroviruses, ALV J is capable of persisting at very low levels in the host; such low levels may not always be detected by currently used routine diagnostic methods. Hence, the real prevalence of ALV J may be actually higher than what is reported (12). Recent reports of myelocytomatosis induced by ALV J or its natural recombinant (ALV B/J) in commercial white leghorn egg-laying flocks in the United States as well as in China are of concern (10,27).

Both ALV J infection profile and transmission pattern in meat-type chickens are comparable to those of other exogenous avian leukosis viruses (ALVs) (16,17,22,25). Infection with ALV J results in one of the following three infection profiles: 1) V+ A−, viremic with no neutralizing antibody against the inoculated parental viral strain (VNAb); 2) V+ A+, viremic with concurrent VNAb; and 3) V− A+, nonviremic with VNAb. Viral persistence is defined as the continued presence of the virus in the host. Most meat-type chickens persistently infected with ALV J are tolerized and seldom develop a VNAb response (V+ A−) (23). However, some persistently viremic chickens may develop a VNAb response against the infecting virus and the virus persists in the host in face of an intact immune system. Hence, chickens classed as V+ A− and V+ A+ are considered to be persistently viremic.

Vertical transmission of ALV J is similar to that of other exogenous ALVs (16,25). However, ALV J horizontal transmission has been reported to be more efficient than that reported for other exogenous ALVs; it results in high levels of viral persistence (8,11,14). Meat-type chickens infected with ALV J during the first 2 wk after hatch generally have high levels of viral persistence (8,26). Anecdotal field evidence and experimental findings suggest that some meat-type chickens infected with ALV J have episodes of ALV J viremia interspersed with variable periods of apparently viremia-free status or *vice versa* (25). This discontinuous viremia might be responsible for the sporadic incidence of ALV J-positive cases in flocks classed as ''ALV J-free,'' and demonstrate failure of some ALV eradication programs. In recent years, the term ''ALV J-tested negative'' has been recommended to use in lieu of ''ALV J-free'' for defining negative flocks (12).

Although several studies have demonstrated the effect of strain and dose of virus and age at infection on subgroup A ALV persistence, antibody response, and oncogenicity (1,4,9,15), data on the effect of such factors on ALV J persistence and oncogenicity are lacking. The objective of the current study was to demonstrate the influence of strain and dose of virus and age at inoculation on ALV J persistence, VNAb response (incidence of VNAb and ability to clear viremia), oncogenicity, and mortality in commercial meat-type chickens.

MATERIALS AND METHODS

Chickens and housing. Fertile eggs from a commercial meat-type breeder were incubated and hatched in isolation at the Avian Disease and Oncology Laboratory (ADOL) in East Lansing, MI. Chickens were inoculated either at the fifth day of embryonation (5 ED) or at day of hatch (DOH). At hatch, chickens were wing-banded and housed in their respective biosecurity level-2 (BSL-2) floor pens. Chickens inoculated with one type of ALV J strain were housed in a self-contained BSL-2 floor pen. Chickens receiving a low dose (100 50% tissue-culture infective dose [$TCID_{50}$]) were separated from chickens receiving a high dose (10,000 $TCID_{50}$) by a 3-foot impervious barrier at the base and wire meshing on top reaching the roof. Forty-five uninoculated negative-control chickens (free of all exogenous ALVs as indicated by the breeder and confirmed by virus isolation) were housed in a separate BSL-2 floor pen. Feed was restricted by alternate-day feeding after 6 wk to limit excessive weight gain, as recommended by breeder. All chickens were cared for and handled according to ADOL animal care and use committee guidelines.

As demonstrated in Table 1, inoculation of chickens with different ALV J strains at various doses at 5 ED or DOH did not always result in 100% infection as detected by virus isolation 1 wk of age. At 1 wk of age, the percent of viremic chickens in groups inoculated with ALV J at 5 ED ranged from 45% to 100%. Except for chickens inoculated with strain ADOL 6803, the percentage of viremic chickens in groups inoculated with 100 $TCID_{50}$ was comparable to that in groups inoculated with 10,000 $TCID_{50}$ of virus. The percentage of viremic chickens in groups inoculated with virus at DOH ranged from 25% to 79%. Except for groups inoculated with ADOL 4817, the incidence of viremia in chickens inoculated with 100 $TCID_{50}$ was comparable to that

Table 1. ALV J viremia in 1-wk-old commercial meat-type chickens following inoculation with 100 or 10,000 $TCID_{50}$ of various strains of virus at 5 ED or at DOH.

Virus	Age at inoculation	Dose ($TCID_{50}$)	No. inoculated	No. hatched[A]	No. infected[B]
ADOL Hc1	5 ED	100	30	15	10
ADOL Hc1	5 ED	10,000	30	19	12
ADOL 6803	5 ED	100	30	12	5
ADOL 6803	5 ED	10,000	30	20	20
ADOL 4817	5 ED	100	30	15	14
ADOL 4817	5 ED	10,000	30	15	14
ADOL Hc1	DOH	100	29	NA	23
ADOL Hc1	DOH	10,000	29	NA	23
ADOL 6803	DOH	100	30	NA	19
ADOL 6803	DOH	10,000	30	NA	20
ADOL 4817	DOH	100	30	NA	8
ADOL 4817	DOH	10,000	30	NA	19

[A]Number of chickens that hatched after 5 ED inoculation. NA = not applicable.
[B]Number of viremic chickens at 1 wk of age as determined by virus isolation test.

Table 2. ALV J infection profile, viral persistence, and VNAb response in commercial meat-type chickens following inoculation with 100 or 10,000 $TCID_{50}$ of various strains of virus at 5 ED or at DOH.[A]

Virus	Age at inoculation	Dose ($TCID_{50}$)	No. of chickens	Infection profile (%)[B]			Viral persistence[C] (%)	VNAb[D] (%)
				V+ A+	V+ A−	V− A+		
ADOL Hc1	5 ED	100	10	0	90	10	90	10
ADOL Hc1	5 ED	10,000	12	0	100	0	100	0
ADOL Hc1	DOH	100	23	17	66	17	83	34
ADOL Hc1	DOH	10,000	22	4	86	10	90	14
ADOL 6803	5 ED	100	4	75	25	0	100	75
ADOL 6803	5 ED	10,000	17	6	94	0	100	6
ADOL 6803	DOH	100	18	22	72	6	94	28
ADOL 6803	DOH	10,000	19	26	74	0	100	26
ADOL 4817	5 ED	100	13	15	85	0	100	15
ADOL 4817	5 ED	10,000	12	8	92	0	100	8
ADOL 4817	DOH	100	6	33	67	0	100	33
ADOL 4817	DOH	10,000	18	6	94	0	100	6
Total[E]				14	82	4	96	18

[A]The statistical differences for the data presented in this table are summarized in Tables 3, 4, and 5.
[B]Viremia and VNAb data were classified into three ALV J infection profiles (V+ A+, V+ A−, V− A+) and expressed as percentages.
[C]Percentages of infected chickens positive for viremia.
[D]Percentages of infected chickens positive for VNAb against the inoculated strain of ALV J.
[E]Total expresses the mean of the groups.

in groups inoculated with 10,000 $TCID_{50}$. The difference in the number of infected chickens (Table 1) and number of chickens for which the data is analyzed (Table 2) is because of loss of some chicks because of nonspecific mortality.

Viruses. Three strains of ALV J, namely ADOL Hc1, ADOL 6803, and ADOL 4817, were used in this study; these strains were selected on the basis of geographic origin, year of isolation, and nucleotide sequence differences. These strains were isolated, over a 4-yr period, from separate meat-type chicken farms located in various regions of the United States. Strain ADOL Hc1 was the first isolate of ALV-J in the United States and is considered to be the American prototype ALV J (8). The other two field strains of ALV J used in this study, ADOL 6803 and ADOL 4817, were isolated from farms with a high incidence of myelocytomatosis (8). Strains ADOL 6803 and ADOL 4817 have 89.2% and 93.3% *env* sequence identity with ADOL Hc1 (24). All viruses were propagated in ADOL line 0 secondary chicken embryo fibroblasts, cells that are resistant to subgroup E ALV (3). The virus titers were determined by limiting dilution in tissue culture by Reed–Muench method (19), and expressed as $TCID_{50}$/ml. The titers for the three strains of ALV-J ranged from $10^{5.5}$ to $10^{6.5}$ $TCID_{50}$/ml.

Experimental design. At various times throughout the experiment, chickens were monitored for ALV J-induced viremia and VNAb response. Chickens infected either at 5 ED or DOH with one strain of ALV J were housed in a self-contained BSL-2 floor pen that was provided with filtered air under negative pressure and contained two compartments to separate groups inoculated with 100 $TCID_{50}$ from those inoculated with 10,000 $TCID_{50}$ of virus. Chickens were inoculated *in ovo* at 5 ED via yolk sac route or intra-abdominally at DOH. Chickens were tested for viremia and VNAb at 1, 3, 7, 11, 15, 19, 23, 27, and 32 wk of age. Chickens were also observed for ALV J-induced gross and microscopic lesions during the experimental period of 32 wk.

Virus isolation. Plasma samples collected during each sampling period were tested for viremia by virus isolation according to the procedures described earlier (9).

Virus microneutralization. Plasma samples from various sampling intervals were tested for VNAb against ALV J using virus stocks that were used to infect the experimental chickens. Virus microneutralization assays were performed as described earlier (9). Samples that had a chromogenic reading of 1 or negative on the p27 group-specific antigen (gsa) enzyme-linked immunosorbent assay (ELISA) readout were considered to be positive for VNAb against ALV J, whereas samples with a chromogenic reading of >1 on the p27 gsa ELISA readout were considered to be negative for VNAb against ALV J.

Pathology. All chickens that died and those that survived the experiment were necropsied. Tissues were fixed in 10% neutral-buffered formalin for microscopic evaluation. All tissues were processed, sectioned, and stained with hematoxylin and eosin.

Data analysis. Viremia and VNAb data from individual chickens from all samplings are grouped into three categories: V+ A−, V+ A+, and V− A+ (Fig. 1). These categories are defined as follows: V+ A−, chickens that tested consistently positive for viremia and with no VNAb response; V+ A+, chickens that remained viremic at the end of the study and were concurrently positive for viremia and VNAb response on at least one occasion; and V− A+, chickens that developed VNAb and lacked viremia by the time the study was terminated. Review of entire viremia and VNAb data from multiple samplings for each bird provided a précis of the course of ALV J infection in that particular chicken throughout the experimental period. Moreover, this method of classification gave precise indication of the relationship between viremia and VNAb data from different sampling intervals for each chicken. Chickens that developed VNAb against the inoculated strain of ALV J did not necessarily clear the viremia (V+ A+). Therefore, chickens of categories V+ A+ and V+ A− were considered persistently viremic. Because this study was primarily aimed at evaluation of viral persistence, only chickens that tested positive for viremia at 1 wk of age were included in the data analysis.

Statistical analysis was performed to test for significance between groups by Sign test for nonparametric data using Statistica® (Statsoft, Tulsa, OK). Statistical significance was reported at the less-than-0.05 level of probability and was approaching statistical significance at 0.05–0.08 level of probability.

RESULTS

Effect of strain and dose of virus, and age of bird at inoculation on ALV J infection. The effect of strain and dose of virus and age of bird at inoculation on the frequency of various infection profiles, viral persistence, and VNAb are summarized in Table 2 and the statistical significance is reported in Tables 3, 4, and 5. Chickens that tested positive for viremia at 1 wk of age and followed through the end of the study were assigned to one of the following three categories: V+ A−, V+ A+, or V− A+ (Fig. 1).

A. Category V+A-

#[1]	B1[2]	B2	B3	B4	B5	B6	B7	B8	B9	Profile[4]
1	+	+	+	+	+	+	+	+	+	V
	-	-	-	-	-	-	-	-	-	VNAb
2	+	+	+	+	+	+	+	ND[3]	ND	V
	-	-	-	-	-	-	-	ND	ND	VNAb
3	+	+	+	+	+	+	+	+	ND	V
	-	-	-	-	-	-	-	-	ND	VNAb
4	+	+	+	+	+	+	ND	ND	ND	V
	-	-	-	-	-	-	ND	ND	ND	VNAb

B. Category V-A+

#[1]	B1[2]	B2	B3	B4	B5	B6	B7	B8	B9	Profile[4]
1	+	+	+	-	-	+	-	-	-	V
	-	-	-	-	+	+	+	+	+	VNAb
2	+	+	+	-	+	+	-	-	-	V
	-	-	-	+	-	+	+	+	+	VNAb
3	+	+	+	+	-	-	+	-	ND[3]	V
	-	-	-	-	+	+	+	+	ND	VNAb
4	+	+	+	+	-	-	+	+	-	V
	-	-	-	+	+	-	-	+	+	VNAb

C. Category V+A+

#[1]	B1[2]	B2	B3	B4	B5	B6	B7	B8	B9	Profile[4]
1	+	+	+	+	+	+	+	+	+	V
	-	-	-	-	-	+	+	+	ND[3]	VNAb
2	+	+	+	+	+	+	+	+	ND	V
	-	-	-	-	+	+	+	+	+	VNAb
3	+	+	+	+	+	+	+	+	+	V
	-	-	-	-	+	+	+	+	+	VNAb
4	+	+	+	+	+	+	+	+	+	V
	-	-	-	+	-	-	-	+	-	VNAb

Fig. 1. Examples of classification of ALV J infection profile into 3 categories based on the viremia (V) and neutralizing antibody (VNAb) against the strain of inoculated parental virus data obtained through the nine sampling intervals over a period of 32 wk starting from 1 wk posthatch. (A) Category V+ A− refers to chickens with persistent viremia without any VNAb against the inoculated virus. (B) Category V− A+ refers to chickens that cleared viremia by the end of the study with efficient VNAb against the inoculated virus. (C) Category V+ A+ refers to chickens with concurrent viremia and VNAb against the inoculated virus during at least one sampling. [1] = chicken number, [2] = sampling intervals, [3] = no data because of death of the chicken before study termination, [4] = infection profile in terms of viremia and VNAb against the strain of inoculated ALV J.

Persistently viremic chickens were identified as chickens that remained viremic, regardless of VNAb response (V+ A+ and V+ A−). The percentage of persistently viremic chickens was high in all the groups and ranged from 83%–100% (Table 2). Development of VNAb did not always lead to viremia-free status, as 18% of the chickens (V+ A+, V− A+) developed VNAb and out of which only 4% were able to clear viremia (V− A+) (Table 2).

Effect of strain of virus on ALV J infection (Tables 2, 3). There are no statistically significant differences between viral strains on viral persistence because most of the chickens were viremic by the time the study was terminated. Strain ADOL 6803 had a statistically significant effect on the incidence of VNAb against the inoculated virus in groups 5 ED at 100 $TCID_{50}$ and DOH at 10,000 $TCID_{50}$. In those groups, chickens developed VNAb at a greater frequency (75% and 26%, respectively) than chickens infected with ADOL Hc1 (10% and 14%) and ADOL 4817 (15% and 6%). However, the presence of the VNAb did not guarantee viremia-free status in the above groups infected with ADOL 6803. There are slight differences, although not statistically significant, in the ability of viral strains to clear viremia (V− A+). ADOL Hc1 was able to clear viremia in a few chickens (groups 5 ED at 100 $TCID_{50}$, DOH at 100 and 10,000 $TCID_{50}$). One chicken infected with ADOL 6803 at DOH at 100 $TCID_{50}$ also cleared viremia. None of the chickens infected with ADOL 4817 were able to clear viremia.

Effect of age at inoculation on ALV J infection (Tables 2, 4). A high incidence of viral persistence was observed at both 5 ED (90%–100%) and DOH (83%–100%) infections. No significant differences in viral persistence were found between the groups. Chickens inoculated at DOH tend to have higher incidence of VNAb than that of chickens inoculated at 5 ED in most groups (ADOL HC1 at 100 $TCID_{50}$ and 10,000 $TCID_{50}$, ADOL 6803 at 10,000 $TCID_{50}$, and ADOL 4817 at 100 $TCID_{50}$). Differences were statistically significant in group ADOL 6803 at 10,000 $TCID_{50}$ ($P < 0.05$) and ADOL Hc1 at 100 $TCID_{50}$ ($P < 0.08$). On the other hand, in chickens inoculated with ADOL 6803 at 100 $TCID_{50}$ and ADOL 4817 at 10,000 $TCID_{50}$, the incidence of VNAb was higher following inoculation at 5 ED than with inoculation at DOH.

Effect of virus dose on ALV J infection (Tables 2, 5). Dose of virus had no effect on viral persistence. High incidence of viral persistence was observed at both 100 $TCID_{50}$ (83%–100%) and 10,000 $TCID_{50}$ (90%–100%) infections. Within all groups, the incidence of VNAb response was higher in chickens inoculated at 100 $TCID_{50}$ than in chickens inoculated at 10,000 $TCID_{50}$. The differences were statistically significant in groups ADOL 6803 at 5 ED and 4817 at DOH ($P < 0.05$), Hc1 at DOH ($P < 0.08$).

Effect of strain and dose of virus and age of bird at inoculation on ALV J-induced tumors. The incidence and spectrum of ALV J-induced tumors in various groups are compiled in Table 6. The incidence of tumors in groups inoculated with ADOL 4817 ranged from 50%–67%, compared with 53%–100% and 44%–100% in groups inoculated with ADOL Hc1 and ADOL 6803, respectively. Chickens infected with ADOL 4817 had a greater variety of tumors than chickens infected with ADOL Hc1 and ADOL 6803. ADOL Hc1 induced myelocytomatosis, renal tumors, and histiocytic sarcomas. In addition to these tumors, ADOL 6803 induced erythroblastosis and hemangiomas. Chickens infected with ADOL 4817 had the greatest tumor spectrum, consisting of fibrosarcomas and rhabdomyosarcomas, as well as the above-mentioned tumors.

Chickens infected with 100 $TCID_{50}$ had higher mean death times than chickens infected with 10,000 $TCID_{50}$ (ADOL Hc1 at 5 ED and DOH, and ADOL 6803 at DOH [$P < 0.05$]) except for group ADOL 4817 at 5 ED. Dose did not seem to have any apparent effect on the tumor spectrum.

Age at infection seemed to influence ALV J-induced tumor spectrum because only DOH-infected chickens developed histiocytic sarcomas unlike chickens infected at 5 ED. Chickens inoculated at 5 ED appear to have higher tumor incidence than chickens inoculated at DOH (ADOL Hc1 at 100 $TCID_{50}$ and 6803 at 10,000 $TCID_{50}$ [$P < 0.05$]) (statistical data not presented).

Chickens categorized as V+ A− had the highest incidence of tumors (71%). These chickens also had the widest tumor spectrum and the shortest mean death time (133 days). The presence of antibody reduced the incidence of tumors, narrowed the tumor spectrum, and prolonged mean death time (Table 7).

Table 3. Effect of strain of virus on ALV J infection profile, viral persistence, and VNAb response.[A]

Age	Dose	Strain	Infection profile[B]			Viral persistence	VNAb
			V+ A+	V+ A−	V− A+		
5 ED	100	ADOL Hc1	a[C]	a	a	a	a
		ADOL 6803	b	b	a	a	b
		ADOL 4817	a	a	a	a	a
	10,000	ADOL Hc1	a	a	a	a	a
		ADOL 6803	a	a	a	a	a
		ADOL 4817	a	a	a	a	a
DOH	100	ADOL Hc1	a	a	a	a	a
		ADOL 6803	a	a	a	a	a
		ADOL 4817	a	a	a	a	a
	10,000	ADOL Hc1	a	ab	a#	a#	ab#
		ADOL 6803	b	b	b	b	b
		ADOL 4817	ab	a	b	b	a

[A]Effect of ALV J strain on infection profile, viral persistence, and VNAb was studied by comparing three different strains of ALV J (ADOL Hc1, ADOL 6803, and ADOL 4817) within groups of age and dose.
[B]Viremia and VNAb data were classified into three ALV J infection profiles (V+ A+, V+ A−, and V− A+).
[C]Test for significance between groups was performed by Sign test using Statistica®. Level of statistical significance <0.05, # indicates level of statistical significance is 0.05–0.08. The same letter within strain groups indicate no statistical significance.

DISCUSSION

Witter and coworkers (25) have reported a high incidence of viral persistence in meat-type chickens infected with ALV J. Results from this study also demonstrate a very high incidence of ALV J persistence (V+ A+ and V+ A−) in meat-type chickens, regardless of strain of virus and dose, and age at inoculation. The high viral persistence of ALV J is in contrast to previous reports on response of egg-type chickens to infection with ALV A (7). Usually chickens infected with ALV A after hatch tend to develop transient viremia followed by an efficient VNAb response that is able to prevent reappearance of viremia. Data from the present study demonstrate that the presence of ALV J VNAb in meat-type chickens following infection with virus at hatch does not necessarily lead to a viremia-free status. The incidence of concurrent viremia and VNAb against the inoculated strain of ALV J (V+ A+) in chickens varied from 0%–75%, depending mainly on strain of virus, followed by viral dose and age at infection. A possible explanation for the relatively high

Table 4. Effect of age at inoculation on ALV J infection profile, viral persistence, and VNAb response.[A]

Comparison between age groups	Within viral strain groups	Within dose groups	Infection profiles[B]			Viral Persistence	VNAb
			V+ A+	V+ A−	V− A+		
5 ED and DOH	ADOL Hc1	100	Δ[C]	Δ	NS	NS	Δ
	ADOL Hc1	10,000	NS	NS	NS	NS	NS
	ADOL 6803	100	*	*	NS	NS	*
	ADOL 6803	10,000	*	*	NS	NS	*
	ADOL 4817	100	NS	NS	NS	NS	NS
	ADOL 4817	10,000	NS	NS	NS	NS	NS

[A]Effect of age at inoculation on infection profile, viral persistence, and VNAb was studied by comparing two groups of age at inoculation (5ED and DOH) within groups of viral strain and dose ($TCID_{50}$).
[B]Viremia and VNAb data were classified into three ALV J infection profiles (V+ A+, V+ A−, and V− A+).
[C]Test for significance between groups was performed by Sign test using Statistica®. * = level of statistical significance <0.05, Δ = level of statistical significance is 0.05–0.08, NS = no statistically significant difference.

Table 5. Effect of viral dose on ALV J infection profile, viral persistence and VNAb response.[A]

Comparison between dose groups	Within viral strain groups	Within age groups	Infection profiles[B]			Viral persistence	VNAb
			V+ A+	V+ A−	V− A+		
100 and 10,000 $TCID_{50}$	ADOL Hc1	5 ED	NS[C]	NS	NS	NS	NS
	ADOL Hc1	DOH	Δ	Δ	NS	NS	Δ
	ADOL 6803	5 ED	*	*	NS	NS	*
	ADOL 6803	DOH	NS	NS	NS	NS	NS
	ADOL 4817	5 ED	NS	NS	NS	NS	NS
	ADOL 4817	DOH	*	*	NS	NS	*

[A]Effect of viral dose on infection profile, viral persistence, and VNAb was studied by comparing two groups of virus dose (100 and 10,000 $TCID_{50}$) within groups of viral strain and age at inoculation.
[B]Viremia and VNAb data were classified into three ALV J infection profiles (V+ A+, V+ A−, and V− A+).
[C]Test for significance between groups was performed by Sign test using Statistica®. * = level of statistical significance <0.05, Δ = level of statistical significance is 0.05–0.08, NS = no statistically significant difference.

Table 6. Effect of strain of virus and dose, and age at inoculation on ALV J-induced tumors.

Virus	Age	Dose ($TCID_{50}$)	Total no. of chickens	Mean number of days until death	Percentage of chickens with tumors	Tumor spectrum[A]
ADOL Hc1	5 ED	100	9	180	100	ML, MB, RT
ADOL Hc1	5 ED	10,000	11	124	91	ML
ADOL Hc1	DOH	100	19	172	53	ML, HS
ADOL Hc1	DOH	10,000	19	161	84	ML, HS
ADOL 6803	5 ED	100	4	181	100	ML, EB, HA
ADOL 6803	5 ED	10,000	17	141	82	ML, EB, HA, RT
ADOL 6803	DOH	100	17	162	71	ML, EB, HA, HS
ADOL 6803	DOH	10,000	18	161	44	ML, HS, HA
ADOL 4817	5 ED	100	12	154	50	ML, FS
ADOL 4817	5 ED	10,000	11	164	64	ML, MB, EB, FS, RT, RMS
ADOL 4817	DOH	100	6	189	67	ML, RT, EB, HS
ADOL 4817	DOH	10,000	16	173	63	ML, EB, HA, RT, HS

[A]Type of tumors diagnosed by gross and microscopic pathology. ML = myelocytomatosis, MB = myeloblastosis, RT = renal tumors, HS = histiocytic sarcoma, EB = erythroblastosis, HA = hemangioma, FS = fibrosarcoma, and RMS = rhabdomyosarcoma.

incidence of V+ A+ status is that ALV J might have mutated and escaped the VNAb response that is directed against the original strain of virus used to infect chickens. The presence of VNAb escape variants have been also been reported to be common in cases of lymphocytic choriomeningitis virus and human immunodeficiency virus infections (2,20). The role of VNAb escape variants in the high incidence of V+ A+ category in ALV J infections was reported elsewhere (13).

The viral strain and dose and age of bird at inoculation seem to have an effect on the incidence of VNAb; however, the differences were statistically significant in only some treatment groups. Chickens infected with ADOL 6803 developed VNAb at a greater frequency than chickens infected with ADOL Hc1 and ADOL 4817. Similar differences in the incidence of VNAb have been reported in infections with different subgroup ALV A strains (6). In addition, slight differences were observed in the efficacy of the VNAb to clear the viremia. Chickens infected with ADOL Hc1 produced VNAb that was able to clear the viremia (up to 17% clearance) better than chickens infected with ADOL 6803 (6%) and ADOL 4817 (0%). Even though the results are not statistically significant, these results suggest that strain of virus may influence not only the incidence of VNAb response but also the ability to clear the viremia. Strain ADOL Hc1 is a laboratory-adapted strain similar to RAV-1 and other ALVs maintained in the laboratory that are known to induce a better VNAb response than field strains (3,6).

In most treatment groups (ADOL Hc1 at 100 and 10,000 $TCID_{50}$, ADOL 6803 at 10,000 $TCID_{50}$, ADOL 4817 at 100 $TCID_{50}$), chickens inoculated at DOH had higher incidence of VNAb than that of chickens inoculated at 5 ED (ADOL 6803 at 10,000 $TCID_{50}$ [$P < 0.05$]; ADOL Hc1 at 100 $TCID_{50}$ [$P < 0.08$]). Unexpectedly, chickens inoculated with ADOL 6803 at 100 $TCID_{50}$ at 5 ED had higher incidence of VNAb than that of chickens inoculated at DOH. Interpretation of these data has to be done cautiously because there were only four chickens in this group (5 ED at 100 $TCID_{50}$). However, development of VNAb in chickens inoculated at 5 ED has been reported in subgroup A ALV by Rubin (21). In Rubin's experiments, white leghorn chickens inoculated *in ovo* with ALV A developed neutralizing antibodies against the inoculated virus (21). He suggested that chickens inoculated *in ovo* may elicit a neutralizing antibody response, especially if the effective inoculated dose is low enough not to cause tolerance (21).

Results from the present study demonstrate that meat-type chickens inoculated with 100 $TCID_{50}$ (34%–75%) had higher VNAb response than chickens inoculated with 10,000 $TCID_{50}$ (0%–26%). These results are similar to that reported for ALV A infections in white leghorn chickens (6).

Conventionally, ALV infection is defined on the basis of the presence or absence of ALV viremia, shedding, and VNAb from single or multiple samplings (22). Based on data (obtained from 20 samplings over a period of 60 wk) on frequency and consistency of viremia and cloacal shedding, Witter and coworkers (25) defined five ALV J infection profiles (consistent, intermittent A, intermittent B, transient, and negative). In the present study, results on ALV J infection status including VNAb for each individual chicken were obtained from nine samplings over a period of 32 wk; these data allowed comprehensive evaluation of viral persistence in VNAb-positive and -negative chickens. Analysis of results obtained only from chickens that tested positive for virus at 1 wk of age eliminated the possibility of skewing data by using results from inoculated, but uninfected, chickens.

Table 7. Effect of ALV J infection profiles on tumor incidence and tumor spectrum observed in the three ALV J infection profiles.

Infection profile[A]	No. of chickens	Mean no. of days until death	Percentage of chickens with tumors[B]	Tumor spectrum[C]
V+ A−	127	133[a]	71[a]	ML, RT, MB, HA, HS, EB, FS, RMS
V+ A+	21	159[b]	48[b]	ML, EB, HA, HS
V− A+	3	195[b]	33[a,b]	HA

[A]Viremia and VNAb data were classified into three ALV J infection profiles (V+ A+, V+ A−, V− A+).

[B]Test for significance between groups was performed by Sign test using Statistica®. Lowercase letters indicate statistically significant differences between groups at the $P < 0.05$ level.

[C]Type of tumors diagnosed by gross and microscopic pathology. ML = myelocytomatosis, MB = myeloblastosis, RT = renal tumors, HS = histiocytic sarcoma, EB = erythroblastosis, HA = hemangioma, FS = fibrosarcoma, and RMS = rhabdomyosarcoma.

Comparison between number of inoculated and infected chickens in groups inoculated at 5 ED or DOH revealed that not all inoculated chickens became infected. A very low incidence of infection was noted in two groups of inoculated chickens, namely 100 $TCID_{50}$ of ADOL 6803 inoculated at 5 ED and ADOL 4817 inoculated at DOH. The reason for failure of 5 ED inoculation to result in 100% infection by 1 wk of age was not determined, but can be explained by several reasons including loss of titer because of the thermolabile nature of the virus, inoculation technique, or errors or limitations of the virus assay methods used in this study.

Analysis of results on tumors revealed that the tumor spectrum induced by ADOL 4817 was more diverse than that induced by ADOL 6803 and ADOL Hc1, suggesting that strain of virus may influence tumor incidence and spectrum. The effects of strain of virus on ALV A-induced tumor incidence and spectrum have been documented (6). In the present study, only chickens inoculated at hatch, but not *in ovo*, developed histiocytic sarcomas, suggesting that age at inoculation may influence the development of these lesions. Chickens inoculated with 100 $TCID_{50}$ lived longer than chickens inoculated with 10,000 $TCID_{50}$, suggesting that dose of virus may influence the mean death time. Virus dose did not have any apparent effect on tumor spectrum, in contrast to the earlier reports with ALV A infections (18). Dose of inoculated ALV has been shown to greatly influence the incubation period for tumor formation as well as the type of tumor produced (18). Experimental studies by Burmester *et al.* have demonstrated that high doses of certain ALV A strains predominantly induce erythroblastosis with a short incubation period of 2–3 mo, whereas low doses predominantly induce lymphoid leukosis (LL) with a relatively longer incubation period of 5–9 mo (1). Under field conditions, ALV exposure is generally at low doses and hence the reason for high incidence of LL (15). Also, congenitally infected chickens have high viral loads but still the incidence of LL is more common than leukemias (15). Therefore, the effect of ALV dose on tumor spectrum is still unclear.

Incidence of VNAb response had a major effect on mean death time and oncogenicity (incidence and tumor spectrum). Presence of antibodies even in chickens that are unable to clear viremia reduced the pathogenicity of the virus. However, the beneficial effects were more evident when the VNAb was able to clear the viremia. Influence of maternal antibodies on response of chickens to infection with ALV A has been documented (5).

Data from the current study demonstrate that infection of meat-type chickens at an early age with ALV J results in a high incidence of viral persistence (V+ A+ and V+ A−). In contrast to ALV A infection, most of the chickens inoculated with ALV J at DOH tend to remain persistently viremic. In addition, the current data clearly demonstrate that the presence of ALV J VNAb does not necessarily lead to a viremia-free status. The data also show that strain and dose of virus and age at infection may also influence ALV J-induced tumor response. This study provides relevant information on ALV J persistence that may aid in ALV eradication procedures.

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